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Elevated ACE activity is not associated with asthma, COPD, and COPD co-morbidity

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Summary
The *angiotensin-converting enzyme* (*ACE*) gene is a potential candidate gene for risk of asthma, COPD, and COPD co-morbidity. In 9034 Danish adults, we determined whether individuals homozygous or heterozygous for the *ACE D* allele are at greater risk of asthma, COPD, or COPD co-morbidity compared with *ACE II* homozygous individuals. In the general population, serum ACE activity increased with the number of *D* alleles (Kruskal-Wallis ANOVA: *II* vs. *ID*, $p < 0.001$; *ID* vs. *DD*, $p < 0.001$); however, this did not translate into altered risk of asthma or COPD. In the general population, the odds ratio (95% confidence interval) for asthma was 1.2 (0.9–1.4) for *ID* individuals and 1.2 (0.9–1.5) for *DD* individuals compared with *II* individuals. In the general population, the odds ratio for COPD was 0.9 (0.8–1.1) for *ID* individuals and 1.0 (0.8–1.2) for *DD* individuals compared with *II* individuals. Among patients with COPD, the odds ratio for ischemic heart disease was 1.1 (0.8–1.6) for *ID* individuals and 1.2 (0.8–1.7) for *DD* individuals compared with *II* individuals; corresponding odds ratios for hypertension were 1.1 (0.7–1.5) and 0.8 (0.5–1.2), and for low physical activity 0.9 (0.5–1.4) and 0.7 (0.4–1.2). The results were similar upon adjustment for sex, age, smoking status, body mass index, total cholesterol, and ACE inhibitor/angiotensin II type 1 receptor blocker use. These data suggest that lifelong genetically elevated ACE activity is not a major risk factor for asthma or COPD, or for ischemic heart disease, hypertension, and low physical activity in COPD patients.

Background

The World Health Organization (WHO) lists chronic obstructive pulmonary disease (COPD) as the fifth most common cause of death, constituting some five percent of the total deaths in 2003.[1] This number is expected to rise in the near future with the current increase in

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tobacco consumption in the third world being of major concern.

COPD is characterized by an abnormal excessive inflammatory response of the lung parenchyma to inhaled irritants and toxins, and by the presence of systemic inflammation.[2] The most important risk factor for development of COPD is tobacco smoking. It is well known that COPD patients often suffer from ischemic heart disease (IHD) and hypertension, and one study suggests that the risk for cardiovascular illness in COPD is associated with severity of the underlying pulmonary disease.[3] Although respiratory failure is the predominant cause of death in patients with advanced COPD (GOLD stages III and IV), cardiovascular disease is one of the main causes of mortality in milder cases. Furthermore, progression of disease in COPD may be promoted by low physical activity, which frequently occurs in COPD patients due to loss of skeletal muscle strength.[4]

The *angiotensin-converting enzyme* (*ACE*) gene is one potential candidate gene for risk of asthma,[5] COPD,[6] and co-morbidity in COPD.[7–9] The primary function of ACE is the conversion of angiotensin I to angiotensin II. This oligopeptide is in itself a potent vasoconstrictor with speculated involvement in the development of primary hypertension,[10,11] however, angiotensin II also mediates biosynthesis of growth factors and might, thus, modulate the development of cachexia,[12] and impaired physical capability in COPD patients.

Clinical trials where modulators of the renin-angiotensin system (ACE inhibitors and angiotensin II type 1 receptor blockers) were administered to COPD patients have indicated that medically reduced angiotensin II synthesis is beneficial in these patients.[7,9] These studies showed not only lowered incidence of cardiovascular co-morbidity, but also reduced hospitalisation and mortality due to COPD. It is therefore likely that genetically altered ACE activity could affect risk of cardiovascular co-morbidity in COPD patients.

A well-characterized variation of the *ACE* gene defined by the absence (Deletion, *D* allele) vs. presence (Insertion, *I* allele) of a 287 base-pair fragment in intron 16 is associated with increased ACE activity,[13–15] and consequently with raised levels of angiotensin II and other vasoactive substances.[15,16]

Using data from The Copenhagen City Heart Study, a prospective general population study, Agerholm-Larsen et al. previously showed that the *ACE* genotype increases ACE activity, but not the risk of ischemic heart disease.[14,17] In the present study, we test the hypothesis that individuals homozygous or heterozygous for the *ACE D* allele vs. *II* homozygotes are at greater risk of asthma, COPD, and/or COPD co-morbidity.

Methods

Participants

We studied individuals who participated in the third examination of The Copenhagen City Heart Study from 1991 through 1994. This prospective general population study includes an almost equal number of women (55%) and men stratified into 10-year age groups from 20 to ≥80 years old. Of 10,049 participants (response rate = 58%), 9034 had *ACE I/D* genotype determined and were Caucasians of Danish descent.[17] All participants gave written, informed consent, and Herlev Hospital, Copenhagen University Hospital and the ethics committee for Copenhagen and Frederiksberg approved the study (Study no. 100.2039/91). Details of the selection procedure, examination program, and study subjects have been presented elsewhere.[18,19]

Asthma

The presence of asthma was defined by an affirmative response to the question: ''Do you have asthma?'' If the definition of asthma assumed asthma medicines or was an affirmative answer to the question ''Does exposure to food, medicine, flowers, animals or anything else cause asthma?'', the results were similar to those presented (Table 1 in supplementary).

COPD

A dry wedge spirometer (Vitalograph; Maids Moreton, Buckinghamshire, UK) was used to obtain FEV_1 and FVC values; the spirometer was calibrated daily with a 1-L syringe. Three sets of values with two measurements differing by less than 5% were obtained. The highest measurements of FEV_1 and FVC were used in the analyses as absolute values and as percentages of predicted values using internally derived reference values based on a subsample of healthy never-smokers.[20,21] We used an equal number of subjects in the subgroups for the calculation of percentage predicted values. COPD was defined as an FEV_1 to FVC ratio of less than 0.7 and FEV_1 of less than 80% of the expected value, excluding those with self-reported asthma (Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages II through IV). Out of the 1259 individuals identified with COPD, 314 had GOLD stage II, 609 GOLD stage III, and 336 GOLD stage IV. If the statistical analyses concerning COPD were stratified according to GOLD stages (Tables 2 and 3 in supplementary), the results were similar to those presented. Individuals with self-reported asthma were excluded from the COPD definition, but had spirometry and all other analyses performed.

Ischemic heart disease

Ischemic heart disease (IHD) diagnoses (ICD8: 410–414 and ICD10: I20–I25) were obtained from the Danish National Hospital Discharge Registry. This registry covers all patients discharged from a hospital in Denmark with IHD as either primary or secondary indication for hospitalisation.

Hypertension

Blood pressure was measured by trained technicians using the London School of Hygiene sphygmomanometer on the left arm after 5 min rest with the subject in the sitting position. The fifth Korotkoff sound was used for diastolic pressure. The fall of the mercury column was set to 2 mm/s. The blood pressure cuff was 12×26 cm, but for subjects with an upper arm circumference of >46 cm, a cuff that measured 15×38 cm was used. Inter-observer variation

was tested and found to be statistically insignificant. Hypertension was defined as systolic blood pressure of $\geq$140 mmHg, diastolic blood pressure of $\geq$90 mmHg,[22] and/or treatment with antihypertensive medication.

Low physical activity

The Copenhagen City Heart Study Leisure Time Physical Activity Questionnaire was used to assess physical activity during leisure time within the past year.[23] Subjects reported whether they were 1) almost completely sedentary or did light physical activity less than 2 h per week (e.g. reading, watching television or movies), 2) did light physical activity 2—4 h per week (e.g. walking, biking, light gardening, light sports), 3) did light physical activity more than 4 h per week or more vigorous activity for 2—4 h per week (e.g. brisk walking, fast biking, heavy gardening, sports that cause perspiration or exhaustion), or 4) did highly vigorous physical activity more than 4 h per week or regular heavy exercise or competitive sports several times per week. Low physical activity was defined as an overall activity level of less than 4 h per week (groups 1 and 2). Supporting the validity of the questions, higher levels of resting heart rate, body mass index, and plasma lipids were associated with report of reduced physical activity during leisure time. The questionnaire is based on a Swedish questionnaire, which has been validated against maximal oxygen uptake in 20—42 year old men (maximal oxygen uptake increased with report of increased physical activity during leisure time).[23]

Other covariates

Smoking status was reported as ''current smoker'', ''ex-smoker'', or ''never-smoker''. Body mass index (BMI) was weight (kg) divided by height squared (m^2).

Laboratory measurements

Serum ACE activity was determined kinetically at 340 nm utilizing the tripeptide *N*-(3-(2-furyl)acryloyl)-*L*-phenylalanylglycylglycine (FAPGG) as the substrate (Sigma, St. Louis, MO, USA). Serum ACE activity was measured in 834 randomly selected subjects[14]; Out of these, 117 had COPD, while 717 had no COPD. Cholesterol was measured enzymatically (Boehringer Mannheim).

The insertion/deletion polymorphism of 287 bp in intron 16 of the *ACE* gene was identified by conventional polymerase chain reaction (PCR) using two primers flanking the site of the insertion.[17] Because the *D* allele in heterozygotes is amplified preferentially, probably due to its smaller size, all samples apparently homozygous for the *D* allele were subjected to a second PCR amplification with an insertion-specific primer.[24]

Statistical methods

Statistical analyses were performed using Stata/SE v.10 (StataCorp LP, 4905 Lakeway Drive College Station, Texas 77845, USA). A *p*-value of <0.05 on a two-sided test was considered significant. Pearson's χ^2-test was used to test for differences in the distribution of percentage of females, smokers, and asthma cases across genotypes. Kruskal-Wallis analysis of variance (ANOVA) was used to test for differences in medians of age, BMI, total cholesterol, lung function, and serum ACE activity between the three genotypes; the Mann-Whitney *U*-test was used for *post hoc* pairwise comparisons between genotypes. Logistic regression analysis was used to calculate odds ratios for all endpoints according to *ACE* genotype. Adjusted odds ratios were obtained by including age, smoking, BMI, total cholesterol, and ACE inhibitor/angiotensin II type 1 receptor blocker use as covariates. Correction for multiple comparisons was by the Bonferroni method.

Results

Clinical characteristics

Clinical characteristics did not differ between subjects with the three different genotypes (Table 1); total cholesterol was slightly higher in *II* than in *ID* and *DD* individuals, however, this result did not reach statistical significance after correction for seven multiple comparisons.

The relative genotype frequencies were 24% for *II*, 50% for *ID*, and 26% for *DD*. This was in accordance with the Hardy-Weinberg equilibrium (χ^2-test: $p = 0.53$) and corresponds well with the findings of others.[25]

Among COPD patients, clinical characteristics did not differ according to the three different genotypes; *p*-values for the distribution of age and FEV_1% predicted became insignificant after correction for six multiple comparisons. As expected, COPD patients were older, more likely smokers, and had consistently lower FEV_1% predicted than those without COPD (data not shown). The high number of COPD subjects may be due to the high prevalence of ever smokers in the population.

ACE genotype and serum ACE activity

Serum ACE activity was measured in a subsample of 834 randomly selected participants and 117 out of these had COPD (Fig. 1). The ACE activity for the three genotypes was found to increase with the number of *D* alleles. Among individuals without COPD ($n = 717$) median activities (inter-quartile range, IQR) were 21 U/L (19—25 U/L) for *II*, 27 U/L (23—32 U/L) for *ID*, and 34 U/L (30—42 U/L) for *DD* individuals (Kruskal-Wallis ANOVA: $p < 0.001$, $p_{II\text{-}ID} < 0.0001$, $p_{ID\text{-}DD} < 0.0001$, $p_{II\text{-}DD} < 0.0001$). The corresponding numbers for COPD patients ($n = 117$), were 22 U/L (20—26 U/L), 27 U/L (24—33 U/L), and 36 U/L (33—40 U/L) (Kruskal-Wallis ANOVA: $p < 0.001$, $p_{II\text{-}ID} < 0.001$, $p_{ID\text{-}DD} < 0.001$, $p_{II\text{-}DD} < 0.0001$).

ACE genotype and risk of asthma and COPD

Among the 9034 study subjects, 602 had asthma and 1259 suffered from COPD. The odds ratio for asthma was 1.2 (0.9—1.4) for *ID* and 1.2 (0.9—1.5) for *DD* compared with *II* individuals (Table 2). For COPD, odds ratios were 0.9 (0.8—1.1) for the *ID* genotype and 1.0 (0.8—1.2) for the *DD* genotype. Adjustment for sex, age, smoking status, BMI,

Table 1 Baseline characteristics of study subjects from the general population.

ACE genotype	All ($n = 9034$)				COPD[a] ($n = 1259$)			
	II	*ID*	*DD*	p[b]	*II*	*ID*	*DD*	p[b]
n	2175	4485	2374		314	609	336	
Females, %	55	56	56	0.89	46	50	51	0.38
Age, years	46 (38–54)	46 (37–54)	46 (38–55)	0.77	50 (43–56)	51 (45–58)	50 (43–57)	0.02[c]
Ever smokers, %	76	75	74	0.35	88	85	89	0.32
Body mass index, kg/m^2	24 (22–27)	24 (22–27)	24 (22–27)	0.47	24 (22–27)	24 (22–27)	24 (22–26)	0.13
Total cholesterol, mmol/L	5.8 (5.1–6.6)	5.7 (5.0–6.5)	5.7 (5.0–6.6)	0.02[c]	5.8 (5.2–6.5)	6.0 (5.3–6.7)	6.0 (5.2–6.6)	0.11
FEV_1, % of predicted	89 (79–99)	89 (79–99)	89 (80–99)	0.78	75 (65–84)	74 (65–84)	76 (68–87)	0.03[c]
Asthma,[d] %	6	7	7	0.24	NR	NR	NR	

Values represent number of individuals, percentages or median (inter-quartile range).
[a] Chronic obstructive pulmonary disease (COPD) = $FEV_1/FVC < 0.7$ and FEV_1% predicted < 80%, excluding those with asthma.
[b] p-values by Kruskal-Wallis ANOVA and χ^2-test.
[c] After correction for multiple comparisons, these results did not reach statistical significance.
[d] Asthma = affirmative to the question ''Do you suffer from asthma?''. ACE = angiotensin-converting enzyme; FEV_1 = forced expiratory volume in one second; *II*, *ID*, and *DD* = non-carrier, heterozygous, and homozygous carrier of the *ACE* insertion-deletion polymorphism, respectively. NR = not relevant as those with asthma were excluded among COPD patients.

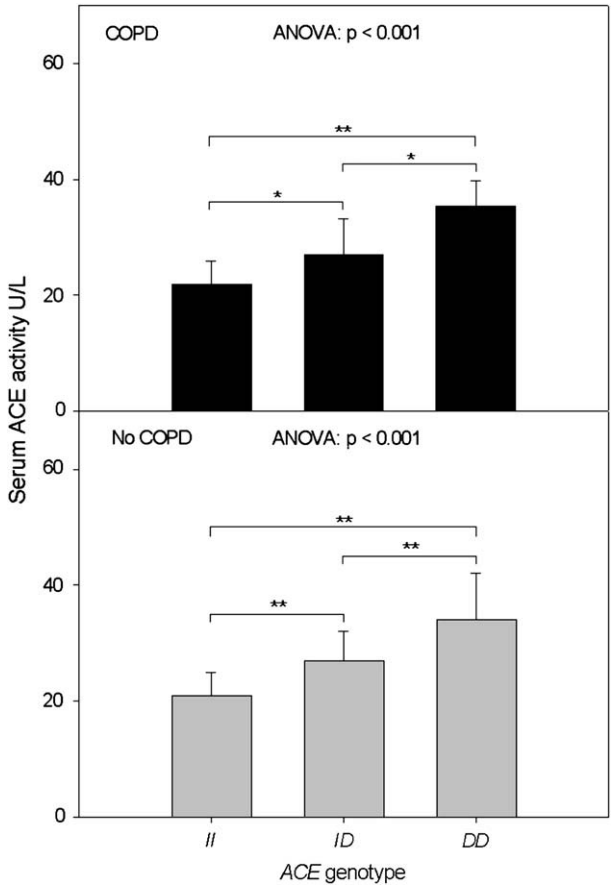

Figure 1 Serum angiotensin-converting enzyme (ACE) activity by *ACE* genotype. *II*, *ID*, and *DD* = non-carrier, heterozygous, and homozygous carrier of the *ACE* insertion-deletion polymorphism, respectively. COPD: $n = 117$; no COPD: $n = 717$; *$p < 0.001$; **$p < 0.0001$.

total cholesterol, and angiotensin-converting enzyme (ACE) inhibitor/angiotensin II type 1 receptor blocker use provided similar odds ratios.

ACE genotype, ischemic heart disease, hypertension, and low physical activity in COPD patients

Among the 1259 COPD patients, 284 had been hospitalised for IHD. The odds ratio (95% confidence interval, CI) for IHD hospitalisation was 1.1 (0.8–1.6) for *ID* and 1.2 (0.8–1.7) for *DD* compared with *II* individuals (Table 3). These results were similar upon adjustment for sex, age, smoking status, BMI, total cholesterol, and angiotensin-converting enzyme (ACE) inhibitor/angiotensin II type 1 receptor blocker use.

Also, of the 1259 COPD patients, 522 were hypertensive. The odds ratio for hypertension was 1.1 (0.7–1.5) for *ID* and 0.8 (0.5–1.2) for *DD* compared with *II* individuals (Table 3). These results were similar upon adjustment for sex, age, smoking status, BMI, total cholesterol, and angiotensin-converting enzyme (ACE) inhibitor/angiotensin II type 1 receptor blocker use.

Finally, of the 1259 COPD patients, 505 subjects had low physical activity level. The odds ratio for low physical activity was 0.9 (0.5–1.4) for *ID* and 0.7 (0.4–1.2) for *DD* compared with *II* individuals (Table 3). These results were similar upon adjustment for sex, age, smoking status, BMI, total cholesterol, and angiotensin-converting enzyme (ACE) inhibitor/angiotensin II type 1 receptor blocker use.

Discussion

It has consistently been found that the *D* allele is associated with increased serum ACE activity[25] and thus, higher levels of circulating vasoactive peptides and inflammatory factors such as angiotensin II, substance P, and neurokinin A. Conceivably, this would imply a role for *ACE* genotype in

Table 2 Risk of COPD or asthma in 9034 individuals from the general population by *ACE* genotype.

ACE genotype	Asthma[a] ($n = 602$)			COPD[b] ($n = 1259$)		
	II	*ID*	*DD*	*II*	*ID*	*DD*
Crude	1.00	1.2 (0.9–1.4)	1.2 (0.9–1.5)	1.00	0.9 (0.8–1.1)	1.0 (0.8–1.2)
Adjusted[c]	1.00	0.8 (0.4–1.4)	0.6 (0.3–1.2)	1.00	1.0 (0.6–1.5)	0.8 (0.5–1.3)

Values represent odds ratios and 95% confidence intervals.
[a] Asthma = affirmative to the question ''Do you suffer from asthma?''.
[b] Chronic obstructive pulmonary disease (COPD) = $FEV_1/FVC < 0.7$ and FEV_1% predicted < 80%, excluding those with asthma.
[c] Adjusted for sex, age, smoking status, body mass index, total cholesterol, and angiotensin-converting enzyme (ACE) inhibitor/angiotensin II type 1 receptor blocker use. *II*, *ID*, and *DD* = non-carrier, heterozygous, and homozygous carrier of the *ACE* insertion-deletion polymorphism, respectively.

pathologies that involve inflammation, such as cardiovascular disease, asthma, and COPD.[6,26–28] Pharmacological studies support a role for ACE in development of cardiovascular co-morbidities in COPD patients. To our knowledge, no larger studies have previously addressed the potential influence of *ACE* genotype on risk of IHD, hypertension, or low physical activity in COPD patients.

Risk of asthma and COPD in the general population

Since *ACE* genotype affects ACE activity and hence modulates the levels of inflammatory factors, carriers of the *D* allele could conceivably experience an excessive inflammatory response to inhaled irritants, and therefore be at higher risk for chronic inflammatory lung disease than non-carriers.

Reports on the involvement of *ACE* in the risk of asthma are somewhat conflicting. The findings are mainly negative, reporting no association of *ACE* genotype with risk or severity of asthma.[28,29] This is contradicted by a French study, which concludes that *ACE DD* genotype is indeed associated with asthma independent of degree of airway obstruction.[5] The bulk of the evidence does, however, point to no association, which is also confirmed in our large study population.

The available literature on *ACE* genotype in relation to risk of COPD is very limited, but in a small study from 2007, Busquets et al. found evidence of an association of *ACE I/D* genotype with smoking history and risk of developing COPD.[6] Our study did not reveal any such association with COPD GOLD stages II, III, and IV analysed separately or combined.

Ischemic heart disease and hypertension in COPD patients

Although previous studies suggest no direct effects of *ACE* genotype on either IHD or hypertension in the general population,[17,24,25] other studies point to potential importance in certain subgroups of individuals. In 2007, Muthumala et al.[26] reported that the *D* allele had a protective effect against development of coronary heart disease in middle-aged hypertensive men through a supposed modulatory effect on systolic blood pressure. However, analogous to the findings for healthy individuals, our results do not indicate any association between *ACE* genotype and risk of cardiovascular complications in COPD.

Low physical activity in COPD patients

Maintaining a moderate level of physical activity is beneficial to the prognosis of COPD. However, physical capacity becomes gradually impaired in individuals suffering from the disease. This is particularly due to quadriceps weakness which is a marked complication of COPD.[30] The effect of *ACE* genotype on exercise capacity has been assessed in both healthy subjects and COPD patients. Intriguingly, the effect of the *D* allele seems to be positively correlated with muscle strength in COPD patients,[8] whereas it has been consistently shown to be negatively associated with muscle strength in healthy subjects.[31,32] A somewhat different mechanism for impaired exercise capacity due to the *ACE DD* genotype is proposed by Kanazawa et al.,[27] who find that this genotype is associated with exaggerated pulmonary hypertension and disturbance in tissue oxygenation

Table 3 Risk of co-morbidity in 1259 COPD patients by *ACE* genotype.

ACE genotype	IHD ($n = 284$)			Hypertension ($n = 522$)			Low physical activity ($n = 505$)		
	II	*ID*	*DD*	*II*	*ID*	*DD*	*II*	*ID*	*DD*
Crude	1.00	1.1 (0.8–1.6)	1.2 (0.8–1.7)	1.00	1.1 (0.7–1.5)	0.8 (0.5–1.2)	1.00	0.9 (0.5–1.4)	0.7 (0.4–1.2)
Adjusted[a]	1.00	1.0 (0.4–2.4)	1.3 (0.5–3.4)	1.00	1.0 (0.7–1.4)	0.9 (0.6–1.3)	1.00	0.9 (0.5–1.4)	0.7 (0.4–1.2)

Values represent odds ratios and 95% confidence intervals.
[a] Adjusted for sex, age, smoking status, body mass index, total cholesterol, and angiotensin-converting enzyme (ACE) inhibitor/angiotensin II type 1 receptor blocker use. IHD = ischemic heart disease; COPD = chronic obstructive pulmonary disease; *II*, *ID*, and *DD* = non-carrier, heterozygous, and homozygous carrier of the *ACE* insertion-deletion polymorphism, respectively.

during exercise. This is also proposed by Gosker et al.,[32] who suggest that exercise capacity, in contrast to muscle strength, is not associated with *D* in COPD, so that the *I* allele is associated with enhanced improvement and/or preservation of endurance and the *D* allele with better improvement and/or preservation of muscle strength.

In our study, we, unfortunately, did not have the means to conduct direct measurements of muscle strength nor to administer exercise tests. Based on the subjects' self-reported physical activity levels during leisure time, we tested for association of the *D* allele with either higher or lower levels of physical activity among COPD patients, but no such associations were found.

Limitations

Some degree of misclassification of asthma was possible, since participants classified themselves; however, the prevalence of asthma was in accordance with the reference figures for Denmark and using alternative definitions of asthma showed similar results. Misclassification of low physical inactivity was also possible. However, supporting the validity of this definition, participants who reported low physical activity also had higher heart rate, body mass index and serum lipid levels. Over the years, the intron 16 *I/D* polymorphism of the *ACE* gene has been the subject of several scientific studies on individuals of various ethnicities. It is speculated that ethnicity is an important factor in determining the magnitude of the effect of genetic variation. The findings of this study are based on a large homogenous population of Danes, all coming from the city of Copenhagen, and the generalisability of our data may therefore be constrained to white people only.

Conclusions

In conclusion, no association was found between the *ACE I/D* genotype and risk of asthma or COPD. *ACE I/D* genotype was not associated with higher prevalence of IHD or hypertension in COPD patients. Likewise, the level of physical activity among COPD patients showed no correlation with *ACE* genotype. The data suggest that lifelong genetically elevated ACE activity is not a risk factor for asthma, COPD, or COPD co-morbidity.

Conflict of interest statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.rmed.2009.04.003.

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